

# **Estimating the Subject by Treatment Interaction in Non-Replicated Crossover Diet Studies**

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# Outline

- History of modeling crossover studies
- Additive main effects, multiplicative interaction (AMMI); LDL-C example
- AMMI variations
- Comparison using a study with some replication
- Results from additional studies
- Conclusions

# History

- First crossover study was started in 1852 to determine if adding N ('ammoniacal salts') improved wheat yield for the alternate years it was administered.
- Diet studies based on a crossover design were first used starting in 1938.
- Early 'switchback' or 'reversal' designs used two sequences, ABAB ... and BABA ...

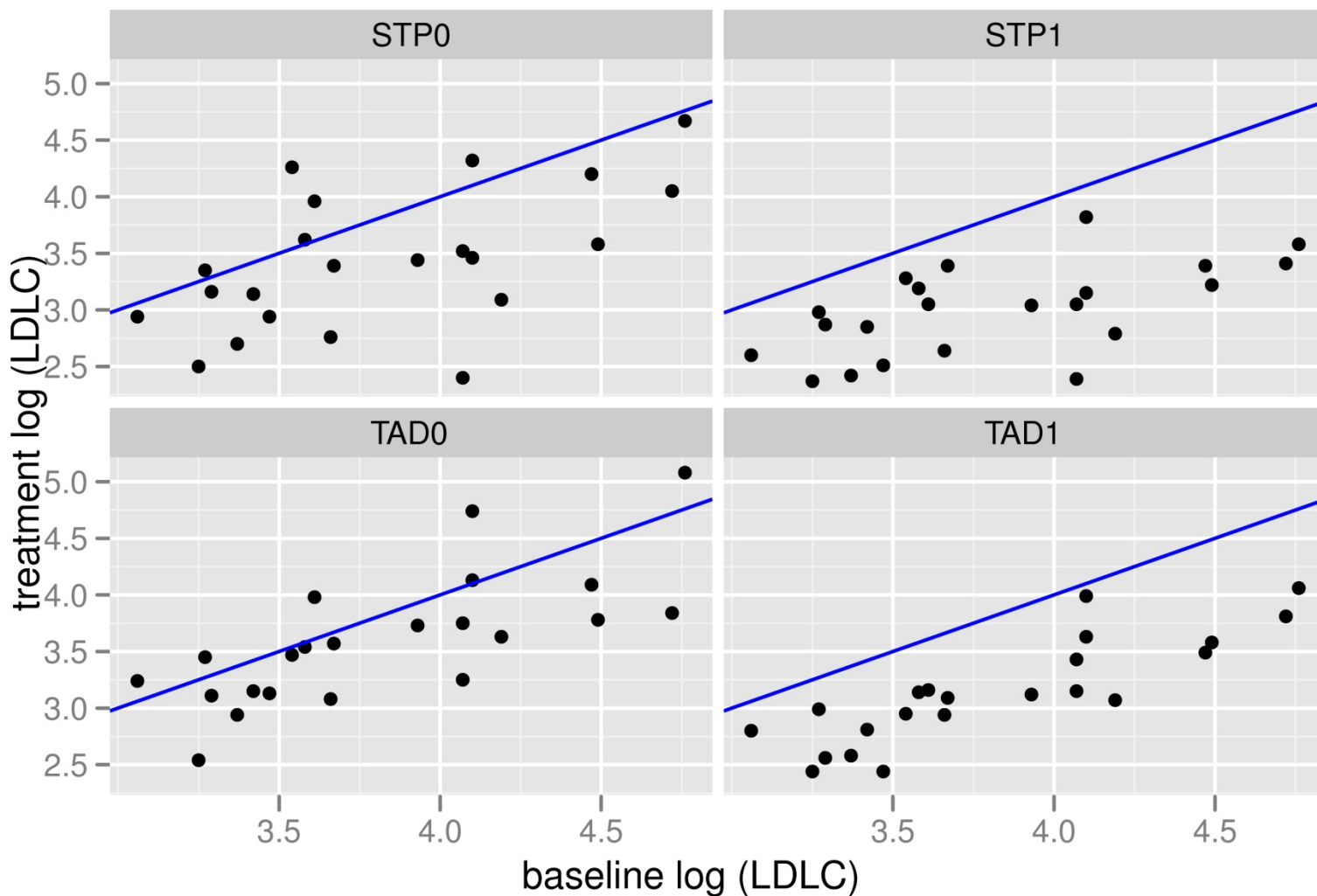
# History

- Early on (1930's and 1940's) statisticians dealt with
  - Carryover effects
  - Period effects
  - Sequence effects
  - Balanced designs (minimizing subjects)
- At some point subjects stopped repeating treatments (why?)

# Consequence of no replication

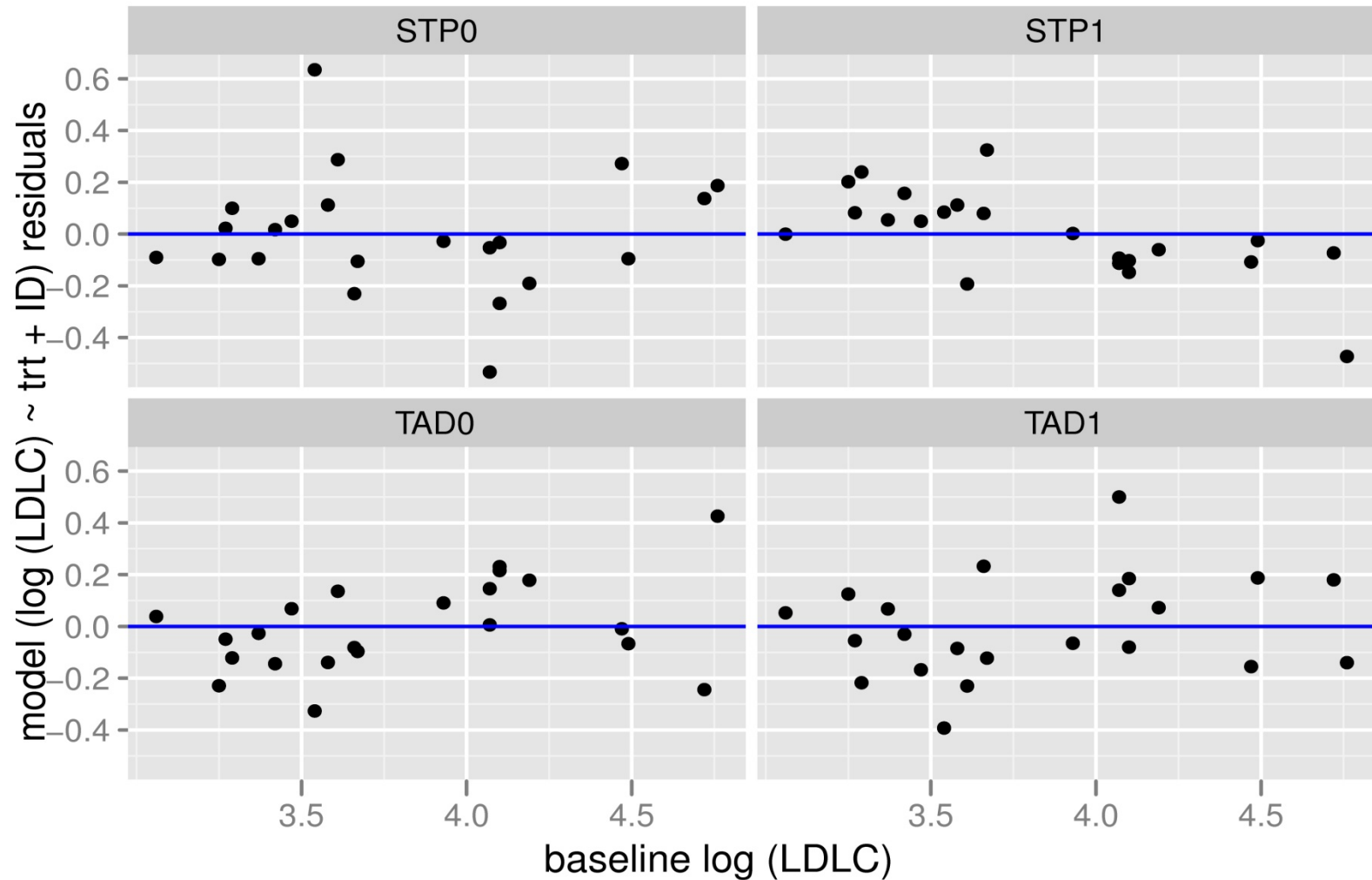
- For normally distributed data, residual and subject by treatment interaction are confounded.
- This isn't a problem if the interaction is small.
- In many studies we looked at, subjects do appear to differ in how they respond to diet, thus there may be an interaction effect.

**STP = Step 1 diet, TAD = typical American diet, 0 = no plant sterols added to diet, 1 = plant sterols added to diet**





# Residuals from model with diet and subject main effects: Is this just noise or is this interaction + noise?



# More History

- Early analyses on crossover results used LM, generally (always?) did not test for a diet by subject interaction, even with subjects repeating treatments.
- If there is an interaction effect, in a LM this is confounded with error, making the residual variance estimate larger, making tests on main effects more conservative. Perhaps not so bad, if the interaction effect is relatively small.
- In 1992 Proc Mixed was introduced, now the subject effect can be random (before that, repeated statement in Proc GLM).
  - Broadens inference space of experiment
  - Usually increases SE of fixed effects, so should make F-tests on diet more conservative
  - DF is an unresolved issue (observations within subject now correlated— leads to a decreased effective sample size)



# More History

- In 2010 at the KSU AgStat conference, ARS statisticians presented results from studying Proc Glimmix, based on simulating data from a complete block design. Reference is Boykin, D., Camp, M.J., Johnson, L., Kramer, M., Meek, D., Palmquist, D., Vinyard, B., and West, M. 2011. Generalized linear mixed model estimation using Proc Glimmix: Results from simulations when the data and model match, and when the model is misspecified. In: Proceedings of the 22nd Annual Conference on Applied Statistics in Agriculture, Kansas State University, April 25-27, 2010. pp. 137-170, available for download at: <https://www.ars.usda.gov/northeast-area/people/matthew-kramer/>
- Goal was to see if there were any special issues one needed to be aware of when using Proc Glimmix to analyze data from simple models.
- The models used contained a fixed treatment, a random block effect, and a random block by treatment interaction.
- Various distributions, including the normal distribution, were used to generate data.
- Of relevance here, they found that F-tests on the treatment effect became too liberal if the random block by treatment interaction was not included in the 'estimating' model.

# More History

- This is the opposite of what happens when all factors are fixed.
- Why does this occur? Since variances of random effects are estimated 'directly', the added uncertainty due to the missing interaction term never finds its way into the test on fixed effects.
- While this has 'always' been known, practicing statisticians and agricultural researchers are largely unaware of the problem.
- In nutrition studies, the de facto standard is to treat subjects as a random effect and ignore the diet by subject interaction (>40,000 studies!).
- Consequence: **Test on diet is too liberal.**
- Problem: There is no replication.

# Methods

- Three approaches to solve the problem.
- One is to cluster 'similar' individuals, and act as though observations from the cluster are repeated measures on one individual. See, e.g. Ghosh and Fairchild (1998) and Ghosh and Crosby (2005). One problem is that groupings change for different dependent variables for the same subjects.
- A second is to fit the confounded interaction + error with a low dimensional model, which will hopefully separate signal from noise.
- A third is to have subjects repeat diets (expensive).

# Separating signal from noise

- Typical approach is to use SVD (principal components).
- This only works if the design is balanced because one needs to form a matrix with treatments as columns and rows as subjects.
- Three variations (there are many more)
  - Traditional AMMI: consider all effects fixed, subtract out main effects, form matrix from residuals, SVD on residuals.
  - AMMI: consider subjects as random effects rather than as fixed effects.
  - gnm package in R (generalized nonlinear models): consider all effects fixed but estimates the model in one call (additional advantages: can test  $i$ th PC for significance, will handle binomial and Poisson data).
- None is ideal but all are better than ignoring the interaction.

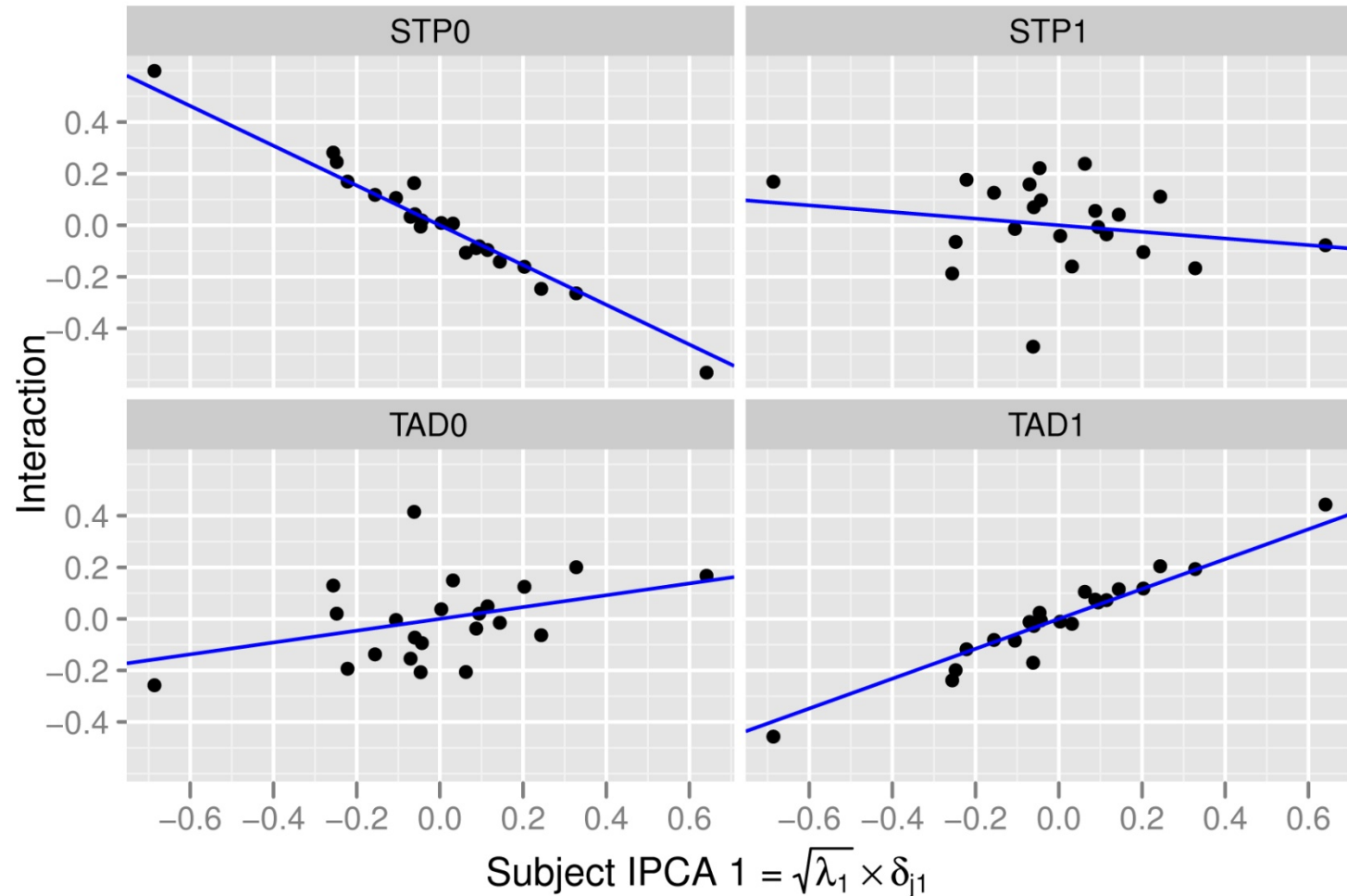
# Model: AMMI with random effects

$$y_{ijk} - (\hat{\mu} + \hat{\beta}x_j + \hat{\tau}_i + \hat{\gamma}_j) = \sum_r \lambda_r v_{ir} \delta_{jr} + \epsilon_{ijk},$$

where  $y$  are LDL data,  $i$  indexes diets,  $j$  indexes subjects,  $k$  indexes diet repeats for subject  $j$ ,  $\mu$  the overall mean,  $\beta$  the slope for the pre-experiment LDL values  $x$ ,  $\tau$  the overall diet effect on LDL,  $\gamma$  the random subject effect,  $\lambda$  the singular value for component  $r$ ,  $v$  the eigenvalue score for diet  $i$  and component  $r$ ,  $\delta$  the eigenvalue score for subject  $j$  and component  $r$ , and  $\epsilon$  random error.

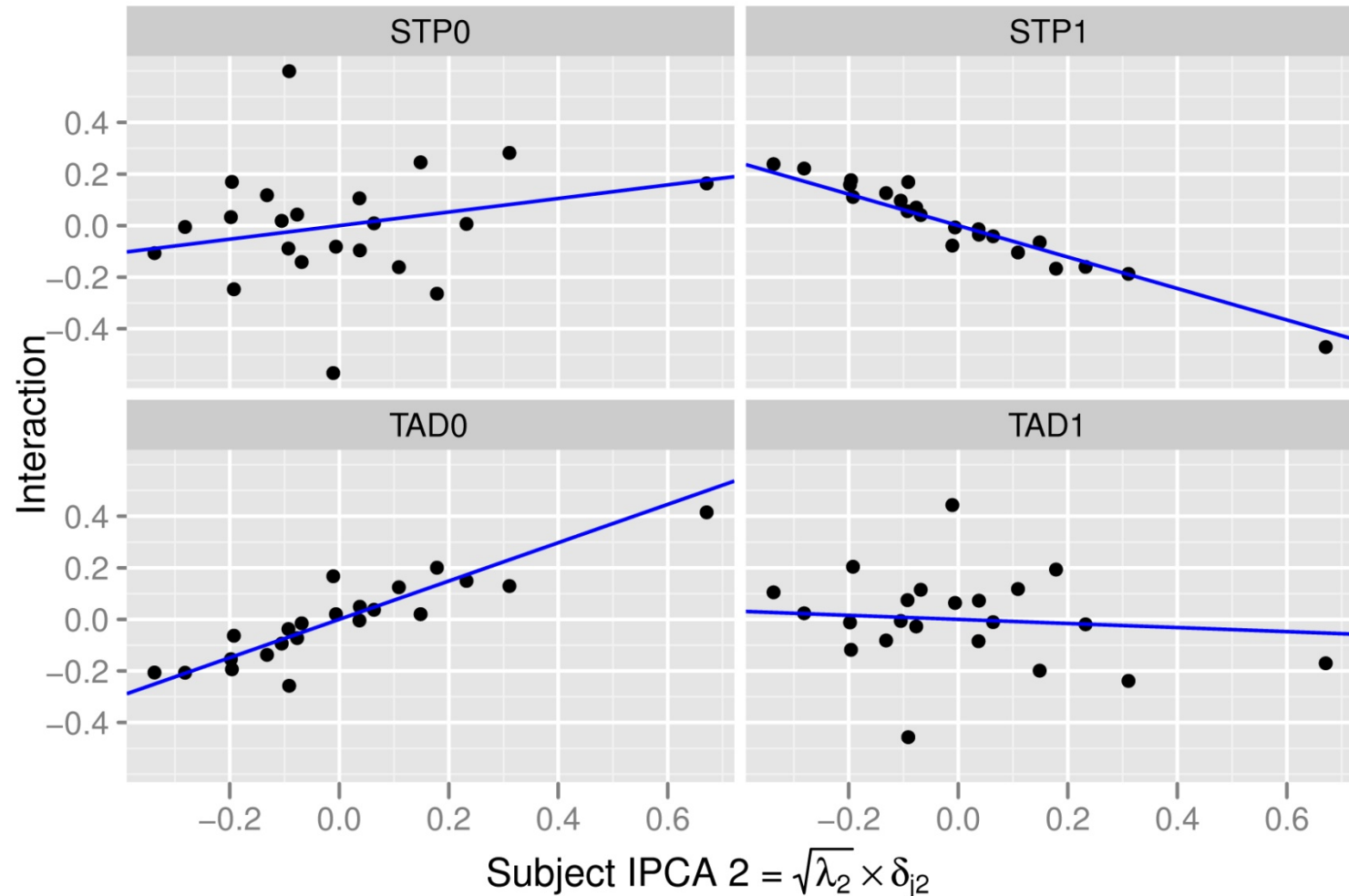


# SVD: Graphical example- PC1





# SVD: Graphical example- PC2



Estimating Subject by Treatment  
Interaction

# LDLC Analysis using gnm in R

```
maineffects1 <- gnm(LDLC ~ trt + ID, data=lp)
bilinear1 <- update(maineffects1, . ~ . + Mult(trt, lp$ID))
bilinear2 <- update(maineffects1, . ~ . + instances
  (Mult(trt, lp$ID), 2))
anova(maineffects1, bilinear1, bilinear2, test = "F")
```

## Analysis of Deviance Table

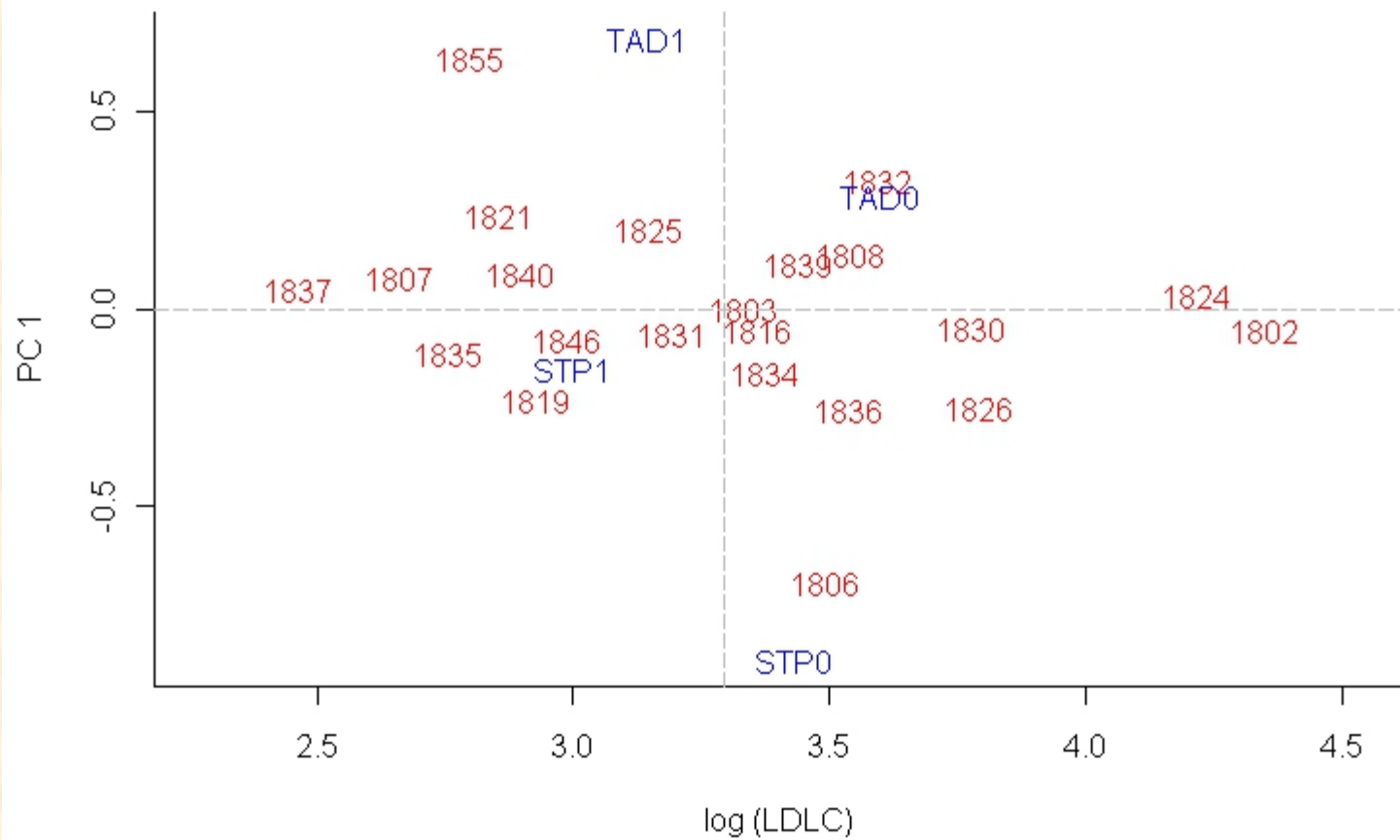
	Resid.	Df	Resid.	Dev	Df	Deviance	F	Pr(>F)	
1		63		3.2190					
2		40	1.3372	23	1.8818	5.3934	0.0002225	***	
3		19	0.2882	21	1.0490	3.2930	0.0057560	**	

# ANOVA Table

**anova (bilinear2)**

	Df	Deviance	Resid. Df	Resid. Dev
NULL			87	27.9587
trt	3	4.8605	84	23.0983
ID	21	19.8793	63	3.2190
Mult(trt, lp\$ID, inst = 1)	23	1.8818	40	1.3372
Mult(trt, lp\$ID, inst = 2)	21	1.0490	19	0.2882

### PC1 vs. Subject and Diet Means



# Traditional AMMI (fixed effects)

- Fit main effects model
- Form a matrix using residuals with treatments as columns and rows as subjects.
- Singular value decomposition of residual matrix.
- Test for the number of 'significant' principal components (e.g. in R you can use the paran function [library(paran)]).
- Calculate df:  $(\text{num. trts} - 1) + (\text{num. subj.} - 1) + (2 * i - 1)$ , where  $i$  is the  $i$ th component.
- SS for  $i$ th component is the square of the  $i$ th value from trace(D) from SVD.
- Create ANOVA table.
- Results are the same as using gnm in R, just more work.

# AMMI (subjects random)

- Fit main effects model, but with subjects random.
- Otherwise as for traditional AMMI.
- Are PCs fixed or random effects? Normally an interaction between a fixed and random effect is considered random.
- If considered fixed, comparison with traditional AMMI:

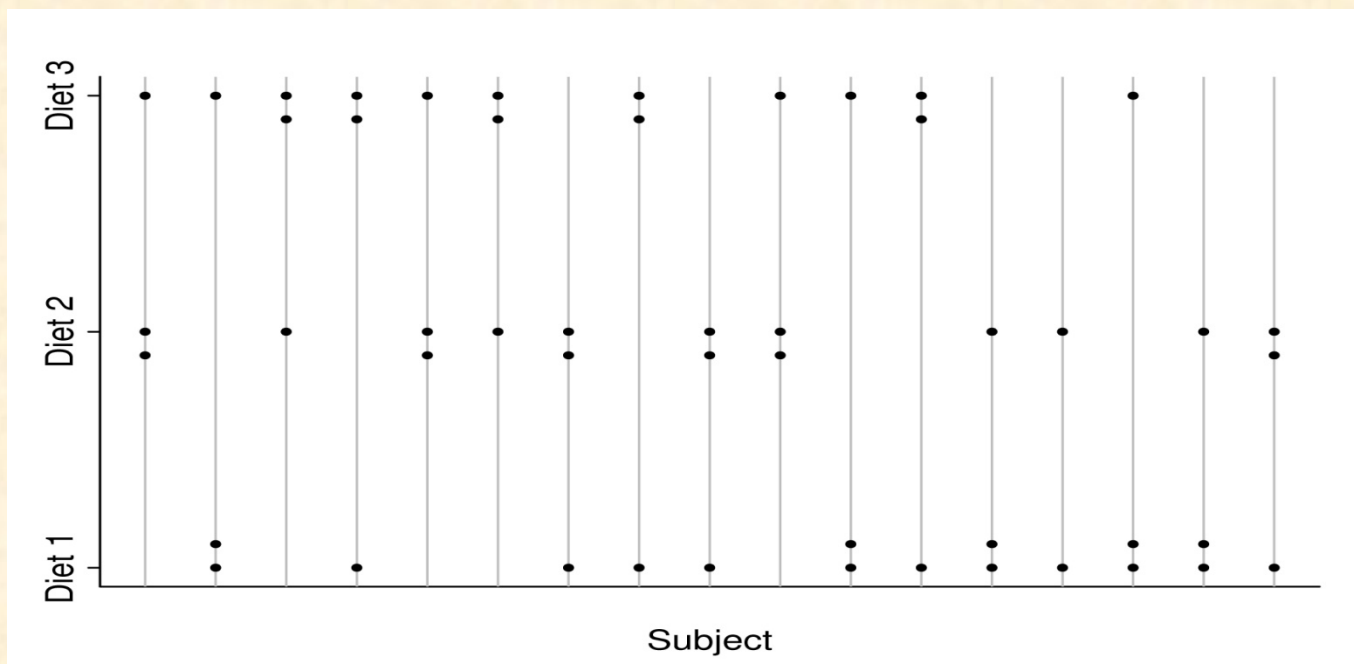
	numDF	SS-RE	SS-gnm
trt	3	4.8605	4.8605
PC1	23	1.8855	1.8818
PC2	21	1.0755	1.0490

Subject  $\sigma^2 = 0.2239$



# Comparison with replicated study

- Looked at effects of including pistachios in diet (control vs. 1 serving vs. 2 servings) on blood components.
- Design: incomplete block, 16 subjects, subjects did two out of three diets, replicating one of the two.



# Comparison with replicated study

- The SVD analysis was performed on three 2-treatment subsets of the data and an augmented data set, where missing values (subject did not participate in one of the treatments) were imputed using a BLUP estimate +  $z_{ij} \sim N(0, \sigma^2)$ , where  $\sigma^2$  is the estimated variance for the subject by treatment interaction effect.
- Repeated diets for a subject were averaged for AMMI analysis.
- Carryover and period effects were small and not modeled.

# Comparison of estimates of interaction effects (subject random)

	Subj × trt variance estimate using mixed model	Variance estimate using PC	Number PCs retained	Number of Subjects
Complete (incomplete block)	0.042599			16
Control vs. 1 serving	0.0037989	0.01049210	1	5
Control vs. 2 servings	0.409760	0.1607147	1	5
2 vs. 1 serving	0.0259188	0.02137756	1	6
Complete + augmented with imputed data	0.040517	0.04634	2	16

# Additional studies

- Two additional data sets from published experiments using a typical crossover design (without replication) were analyzed retrospectively using the multiplicative subject by treatment interaction decomposition.
- Of the 26 variables analyzed in these studies, 19 had large (significant) subject by diet interaction contributions.

# Conclusions

- The subject by treatment interaction is typically important for many variables examined, so should be included in the analysis of human nutrition studies.
- A multiplicative decomposition adequately captures the subject by treatment interaction, and is clearly preferable to ignoring this interaction when analyzing these kinds of data.

# Reference

Kramer, M., S.C. Chen, S.K. Gebauer, and D.J. Baer. 2012. Estimating the subject by treatment interaction in non-replicated crossover diet studies. In: Proceedings of the 23rd annual Kansas State University Conference on Applied Statistics in Agriculture, Manhattan, Kansas, Weixin Yao (ed.). Pp. 96-110.

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